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DISSERTATION SUMMARY

Characterisation of anticryptococcal Fc-1 toxin of *Filobasidium capsuligenum*

Andrea Keszthelyi

Department of Microbiology, University of Szeged, Szeged, Hungary

The basidiomycetous yeast *Filobasidium capsuligenum* (IFM 40078) produces a killer toxin (FC-1) which is highly effective against the opportunistic fungal pathogen *Cryptococcus neoformans*. The sensitivities of strains representing eight molecular subtypes (VNI-IV and VGI-IV) of the *C. neoformans* species complex, and of an additional 50 clinical and environmental isolates were determined. Additionally further yeast species were tested for toxin sensitivity, but FC-1 showed strong specificity for *Cryptococcus* isolates, and did not affect the other examined yeast species. This highly specific effect is mainly characteristic to proteins. Several other observations such as the high thermolability of FC-1, the partial loss of activity in presence of proteases and the considerably narrow pH optimum (pH 4-6) also confirm the protein character of the toxin. Our goal was to characterise and clone the toxin encoding gene and to study the effect of the toxin on *C. neoformans* cells.

Neither RNA, nor DNA plasmids were detectable by agarose gel electrophoresis or pulse-field gel electrophoresis (OFAGE), thus the gene encoding the toxin is most likely located in the chromosomal DNA.

Growing of *Filobasidium capsuligenum* (IFM40078) on complete media leads to toxin production, however on minimal media no biological activity can be detected. In order to identify the toxin the extracellular enzyme profiles of the fungi were determined by polyacrylamide gel electrophoresis in both conditions. Comparison of the results showed that there are a large number of differences between the two profiles making it difficult to unambiguously identify the toxin. Similar observations were perceived when profiles of non-producing *Filobasidium capsuligenum* strain (VKM1513) or non-producing mutant strains generated by UV mutagenesis were compared to the toxin containing profile of strain IFM40078.

Results of competition assay suggest that β-1,6-glucan (pustulan) in the cell wall may provide the binding site for the killer protein. Taking advantage of this specific binding β-1,6-glucan was applied for affinity chromatography as a ligand to purify the toxin. Biologically active fractions from the pustulan-coupled epoxy-activated sepharose 6B column were loaded on polyacrylamid gel. A few proteins in the range of 19 - 51 kDa were detected. A 47 kDa protein, the best candidate for being the toxin, was sequenced (XVNVNGVPI). This sequence could be used for aquiring the coding DNA by reverse transcription.

Other, already characterised killer toxins have been used for homology searches to find conservative amino acid or nucleic acid regions. However no significant homology has been found among these proteins suggesting that such comparative studies do not serve as useful tools for further characterisation of the toxin.

An effective approach for identifying the coding DNA can be the analysis of the toxin producing (IFM40078) and non-producing (VKM1513) strains at the mRNA level. By subtractive hybridisation cDNA segments differing in the two strains can be selectively amplified in order to find the toxin encodig gene.

To investigate the mechanism of the effect of the FC-1 toxin-treated cells were studied. Analysis of cellular DNA by laser scanning cytometry and FITC staining revealed that the killing mechanism of FC-1 is neither cell cycle- nor cell wall biosynthesis-dependent; rather it might act as an ionophor that disrupts the cytoplasmic membrane function.

These various research tools could lead to the complete characterisation of the FC-1 anti-cryptococcal toxin which has the potential to be applied as a therapeutic agent for the treatment of cryptococcosis.

Supervisor: Judit Kucsera
E-mail: keszthelyiandrea@freemail.hu